HORMONES AND TISSUE COMPETENCE IN THE DEVELOPMENT OF DROSOPHILA

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The larvae of Drosophila molt twice, and change with the third molt into the pupal stage, during which the larval organism is gradually made over into the final adult insect. It was found (Bodenstein, 1936) that the initiation of pupation depends upon some factor in the anterior part of the larva which becomes active shortly before pupation and which is presumably hormonal in nature. Hadorn (1937) has located and analyzed this factor more precisely. He brought forward conclusive experimental evidence that a hormone causing pupation in Drosophila is produced by the ring gland, a small organ of internal secretion situated dorsally between the two brain hemispheres of the larvae. Although responsible for pupation, the ring gland was seemingly unable to initiate further pupal development, i.e. the differentiation of the larval organ anlagen to imaginal completion. For larval abdomens which, as a result of the removal of the anterior part, remain constantly larval could be caused to pupate when one or more ring glands were transplanted into them (Hadorn and Neel, 1938). Yet only puparium formation but no further development could be induced. Likewise, transplantation of several ring glands into younger larvae brought about only precocious puparium formation but again no subsequent development (Hadorn and Neel, 1938). In the light of these facts it appeared highly probable that some other hormone than that for puparium formation governed imaginal differentiation. The following observations seem to verify this assumption. The imaginal differentiation of pupal abdomens proceeds to imaginal completion when the anterior pupal part is cut off about 20 hours after pupation but the abdomen remains pupal when the anterior part is removed earlier (Bodenstein, 1938 and 1939a). The imaginal differentiation of organ anlagen, for example, eye discs, also depends upon this factor in the anterior part. On the basis of this rather indirect evidence a special differentiation hormone was postulated (Bodenstein, 1938). However, attempts to localize this factor in the anterior pupal part failed completely (Bodenstein, 1939a and c). Pupal abdomens, the anterior part of which was cut off before the imaginary hormone was released and which consequently were expected to remain pupal, continue their development to imaginal completion when placed in a pure oxygen atmosphere (Bodenstein, 1939c). This observation made the existence of a special hormone for differentiation very doubtful. Moreover it was shown (Bodenstein, 1939c), that the inability of the pupal abdomen to develop could be correlated with disturbances in the development of the tracheal system. These experiments, then, indicated that abnormalities in the functional development of the tracheal system rather than the lack of a special hormone was the cause of the inability of the abdomens

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to develop. While these considerations do not disprove the existence of a differentiation hormone completely, they make its assumption quite unnecessary. The main object of this paper is to bring forward more conclusive evidence that the ring gland is responsible not only for pupation but also for differentiation. Actually we have to consider pupation as the first step in the process of imaginal differentiation.

Material and Methods

The experiments reported here were performed on *Drosophila melanogaster* (Orc. R+) and *Drosophila virilis* (wild stock). Both of these species were used as donors and hosts. Various organ discs were transplanted into the body cavity of adult flies and the development of the transplant in its new environment was studied. This new method (see Bodenstein, 1943b, in press) of using the body cavity of adult flies as a culture medium for larval tissues proved to be very successful and was used throughout this investigation. The mortality rate in these experiments was negligible. All the experimental animals were kept at a constant temperature of 25° ± 0.5° C.

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Experiments

When larval eye or leg discs of Drosophila are transplanted into the body cavity of adult flies, the grafted organ is unable to develop. Although left for many days in the adult environment the graft remains unchanged as far as its morphological appearance is concerned. This observation is in agreement with earlier experiments of this kind (Bodenstein, 1938, p. 497). From this it was assumed that the adult environment is a medium unsuitable for the development of larval organs. It was therefore rather unexpected when it was found that larval eye discs which were transplanted simultaneously with two ring glands of mature larvae into adult flies had grown well beyond their original size. This experiment was repeated as follows: Eye discs of melanogaster donor larvae of equal age were transplanted either alone or together with ring glands into adult melanogaster hosts. Three days after the operation the grafted eye discs were dissected and compared. It was found that the eyes in hosts with ring glands were larger by far than those which were in hosts without ring glands. A great number of similar experiments was then performed, consisting of 243 cases where organ discs were transplanted together with ring glands into adult hosts and 156 control cases where the organ discs were transplanted alone. The bulk of this material comprises many different series; the series varied as to the time the organs were allowed to remain in the adult host, the number of ring glands transplanted into one host, the kind of organ disc used (eye and leg) and the kind of host used (melanogaster and virilis). In comparing the discs in hosts with and without ring glands it was invariably found that the organ discs in the hosts with ring glands had become much larger than the control discs in the hosts without ring glands. Further proof for the initiation of growth by the ring gland was obtained by experiments in which the two partners of a single

organ pair were compared one with the other. For this a pair of eye or leg discs was dissected from a single donor larva and one partner disc transplanted into one host together with two to four ring glands, and the other partner transplanted alone into a second host. The results of these experiments consisting of 34 individual pairs are summarized in Table I, where it can be seen

TABLE I

Paired transplantation of eye and leg discs into two adult hosts. One host receives disc alone, while other receives partner disc and two to four ring glands.

Transplant	Number of pairs	Days pairs remain in hosts	Number of pairs where the disc is larger in hosts with ring glands
mel. eye	3	2	3
mel. eye	1	3	1
mel. leg	4	3	4
mel. eye	3	4	3
viril. leg	2	4	2
mel. leg	2	5	2
viril. leg	8	5	8
mel. eye	1	6	1
mel. eye	1	8	1
mel. leg	1	8	1
viril. leg	3	9	3
viril. leg	1	12	1
viril. leg	1	14	1
viril. leg	3	16	3

that in each pair the disc which was transplanted together with ring glands had become larger than its partner. Figure 1 (*a*, *b*) illustrates very clearly the enormous size difference between two partner discs which were dissected and photographed five days after the operation. This particular pair is a melanogaster leg pair. One partner disc (*a*) was transplanted alone and the other

PLATE I

FIGURE 1. Melanogaster leg disc pair five days after the operation. One partner disc (*a*) was transplanted into an adult melanogaster host; the other partner disc (*b*) into an adult melanogaster host together with four ring glands. Note: the enormous difference in size between the two discs.

FIGURE 2. *b*: virilis leg disc four days after the operation, showing the first signs of metamorphosis, i.e. the beginning of evagination. The disc was transplanted together with four ring glands into an adult virilis male host. *a*: Virilis leg disc at the time of transplantation. *c*: a normal pre-pupal leg disc beginning to evaginate. Note: the similarity in the process of evagination between the transplanted (*b*) and the normal (*c*) leg.

FIGURE 3. A virilis leg disc transplanted together with three ring glands into an adult virilis female host, 13 days after the operation. The leg disc is completely differentiated. Note: the well-formed dark brown chitinous tarsus segments, with hairs and claws well developed.

FIGURE 4. Virilis leg disc pair transplanted into two adult virilis male hosts six days after the operation. Note: same size of both discs.

FIGURE 5. Virilis leg disc pair four days after the operation. One partner disc (*a*) was transplanted into an adult virilis male host and the other partner disc (*b*) into an adult virilis female host. Note: disc in female host (*b*) has become much larger than the partner disc (*a*) in the male host, which has not grown at all.

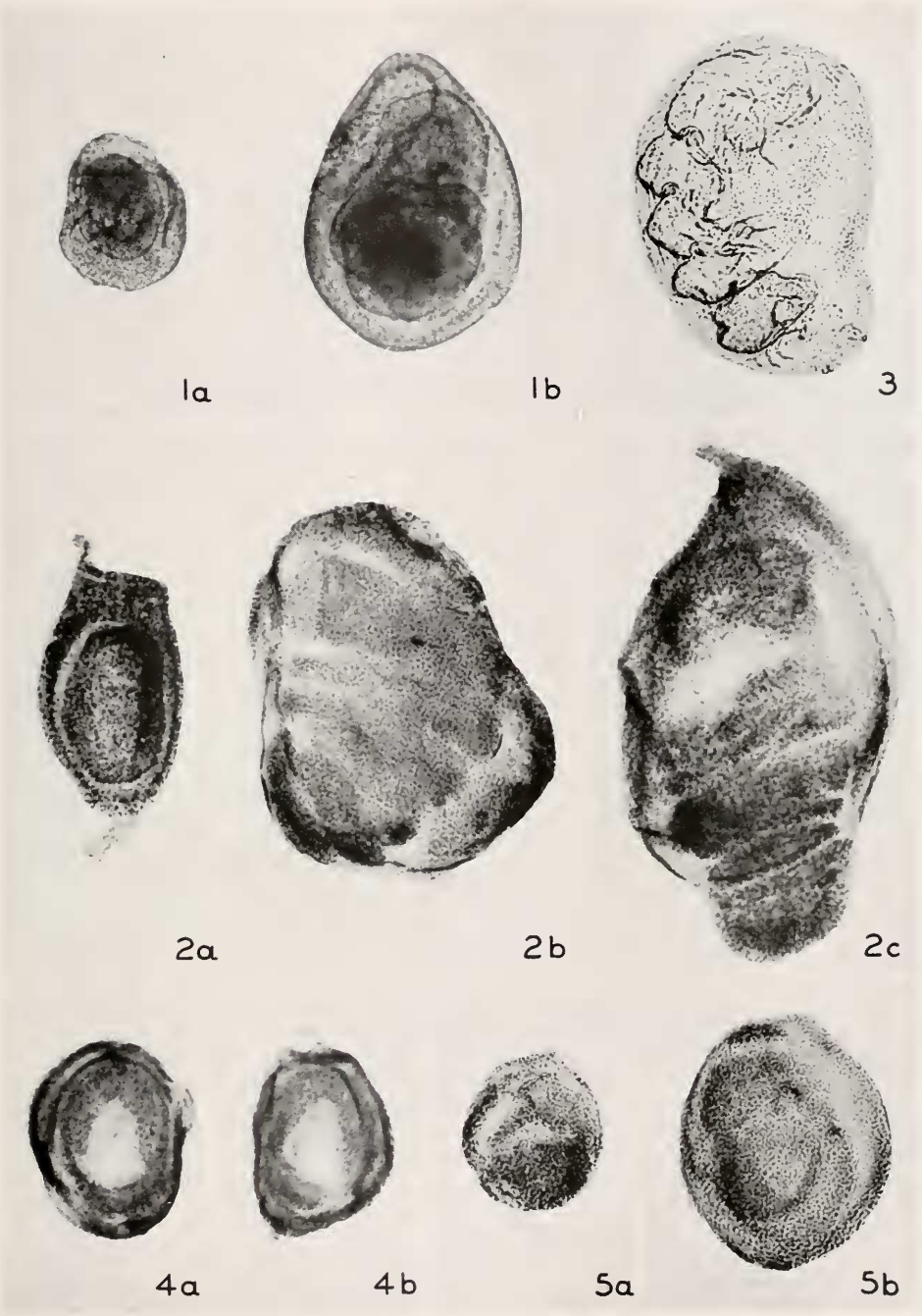

PLATE I

partner (*b*) together with four ring glands into the abdomen of adult melanogaster hosts.

In normal development we notice that a short time after puparium formation many organ discs undergo a characteristic change of form; they evaginate and obtain thus their typical pupal shape. This evagination process is one of the first visible signs of metamorphosis of the organ discs. Now we find that the transplanted leg discs in the body cavity of adult flies also evaginate under the influence of the ring gland after they have grown to a certain size. This induced evagination process is not quite complete, presumably because of mechanical difficulties, but is nevertheless very clear. This is illustrated in Figure 2 (*a*, *b*), Figure 2*a* shows a leg disc at the time of transplantation; Figure 2*b* a leg disc of a normal young pupa which has started to evaginate, and Figure 2*c* a disc which was transplanted into an adult host together with ring glands and which was dissected four days after the operation. In comparing Figure 2*b* with Figure 2*c* one may notice the similarity between normal and induced evagination. This observation clearly proves that the ring gland is able to induce the first stages of metamorphosis in the transplanted leg anlage. Moreover the ring gland is able to induce complete metamorphosis in the leg, if the leg is left in the adult host long enough. In these cases we find a completely differentiated imaginal leg with femur, tibia and tarsus segments as well as well-formed and dark brown chitinized hairs, bristles and claws in the abdominal cavity of the fly (Figure 3). From these experiments it becomes evident that the ring gland is not only responsible for an early initiation of growth, but also for the imaginal differentiation of the organ discs.

While it is true that organ discs transplanted together with ring glands were always larger than the control discs transplanted without ring glands, there was nevertheless a certain variability in the growth of the discs. In some cases where the discs were transplanted alone into adult hosts it was found that they had not grown at all, although they had remained for ten days or longer in these hosts. In other cases the discs had grown quite well and even showed signs of metamorphosis although no ring glands were present. Similarly, discs of equal age grown for the same length of time in the presence of the same number of ring glands could vary quite extensively in size. Now it has to be realized that in experiments of the kind described one deals with three different developmental systems which together determine the outcome of the experiment. These systems are: 1. The adult host environment; 2. The activating system, i.e. the ring glands; 3. The reacting system, i.e. the test organ discs. Thus in order to clarify the observed discrepancies in the experiments, a more thorough investigation of these three systems was undertaken. For this purpose experiments were designed in such a way that two of the systems were held constant and the third one varied. In doing this for each system in turn a clear understanding of the part played by each system was obtained.

I. The adult host environment

Under this general heading we will discuss a number of experiments in which the adult flies used as hosts were varied. As an indicator for possible differences between the various hosts we used only the early growth reaction of the test organs. This method is very sensitive, for even small differences reflect them-

selves very clearly in the growth of the test organ discs, especially when the two partners of a single organ pair are compared.

a. Growth variability test in male hosts without ring glands.

This series was designed to test whether there is any difference in the growth of test organs in different host individuals of the same sex. For this purpose, pairs of leg discs were dissected from virilis larvae and one partner disc transplanted into one and the other partner disc into a second adult virilis male host. The two hosts were then reared and dissected together. From eight such pairs two pairs were dissected two days, one pair three days and five pairs five days after the operation. The partner discs in all pairs were found to be the same size (Figures 4*a* and 4*b*). This proves that there is no detectable difference in the environment of the different individual male hosts as far as the test organs are concerned. Moreover, the leg discs remained unchanged in size, which indicates that no growth had occurred from the time of transplantation until they were dissected five days later. In order to obtain more information on this point, four of these disc pairs were again transplanted into adult male hosts. Seven days later they were dissected and found to be unchanged. Thus we must conclude that the virilis organ discs are unable to grow in an adult virilis male environment.

b. Growth tests in hosts of different age.

The question whether there is any difference in hosts of different age has been tested in the following way. The two partners of a pair of virilis leg discs were transplanted, one into a one-day old virilis male host and the other into a 29-day old virilis male host. From five such pairs one was dissected four days, and four pairs six days after the operation. In all cases it was found that the partner discs of the single pairs were of the same size. There was also no growth in either partner discs during the time they remained in the hosts.

In a second series consisting of six pairs, the discs were transplanted into female hosts instead of into male hosts. One female host was two days old and the other 30 days old. One pair, dissected three days and five pairs dissected five days after the operation revealed again that the two discs of one pair were of equal size. Yet in contrast to the previous series each of the discs had grown during the time it remained in the host.

These experiments prove that there is no difference between young and old hosts. They confirm the previous observation that the environment of each individual male host is the same, and extend the information in showing that this is also true for the environment of each individual female host. The observation that no growth takes place in discs transplanted into male hosts is also confirmed. However, when one compares female and male environment one finds the discs able to grow in the former but not in the latter environment.

c. Growth tests in male and female hosts.

It is evident from the foregoing experiments that male and female environments are different as to their effect on the growth of the grafted organ discs. Decisive evidence for this is provided by the following experiments. The two

partners of the virilis leg disc pairs were transplanted, one into a male, the other into a female virilis host. Five such pairs were dissected three days, and eight pairs four days after the operation. In all pairs it was found that the discs in the female hosts were much larger than their partner discs in the male hosts, which had not grown at all. Figure 5 (*a*, *b*) illustrates this effect very clearly.

Similar results were obtained in another series of experiments (five pairs dissected three days after the operation), where leg discs pairs of virilis were transplanted into female and male melanogaster hosts.

In transplanting the two partners of virilis eye disc pairs into virilis male and female hosts (three pairs dissected three days after the operation), we again find the discs in the female hosts larger than their partners in the male hosts (Figures 6*a* and 6*b*). The eye discs in the male hosts had ceased to grow, being of the same size at the time of dissection as at the time of transplantation.

Finally, in a last series of this kind, the two partners of a pair of salivary glands of virilis larvae were transplanted, one into a male and the other into a female virilis host. From eight such pairs three were dissected in four days, two, seven days and three, eight days after the operation. Again, as in the case of the organ discs, it was found that the glands in the male hosts had not developed while their partners in the female hosts were all in an advanced stage of development.

d. Growth test in different host species.

The object of this group of experiments was to test for possible species differences between virilis and melanogaster hosts. To this end the two partner discs of a virilis leg pair were transplanted, one into a virilis male host and the other into a melanogaster male host. From six pairs available, two were dissected three days, two, four days and two, six days after the operation. The transplanted discs were found to be of the same size in both hosts in all pairs. There is evidently no difference between the melanogaster and virilis environment, as far as it affects the graft.

In a second series of experiments, comprised of seven pairs, which were dissected three days after the operation, the two virilis leg pair partners were transplanted into a melanogaster and a virilis female host. Being in a female environment, the discs in both hosts had, of course, grown; in one pair the discs were of the same size, in three pairs the discs in the melanogaster hosts were

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FIGURE 6. Virilis eye disc pair three days after the operation. One partner disc (*a*) was transplanted into an adult virilis male host and the other partner disc (*b*) into an adult virilis female host. Note: disc in female host has become much larger than partner disc in male host, which has remained unchanged.

FIGURE 7. Virilis leg disc pair three days after the operation. One partner disc (*a*) was transplanted together with two ring glands into an adult virilis male host and the other partner disc (*b*) was transplanted together with two ring glands into an adult melanogaster male host. Note: disc (*b*) in the melanogaster host has become much larger than its partner (*a*) in the virilis host.

FIGURE 8. Virilis leg disc pair four days after the operation. One partner disc (*a*) was transplanted into an adult virilis male host together with four ring glands and its partner (*b*) into an adult virilis male host together with eight ring glands. Note: both discs have grown the same.

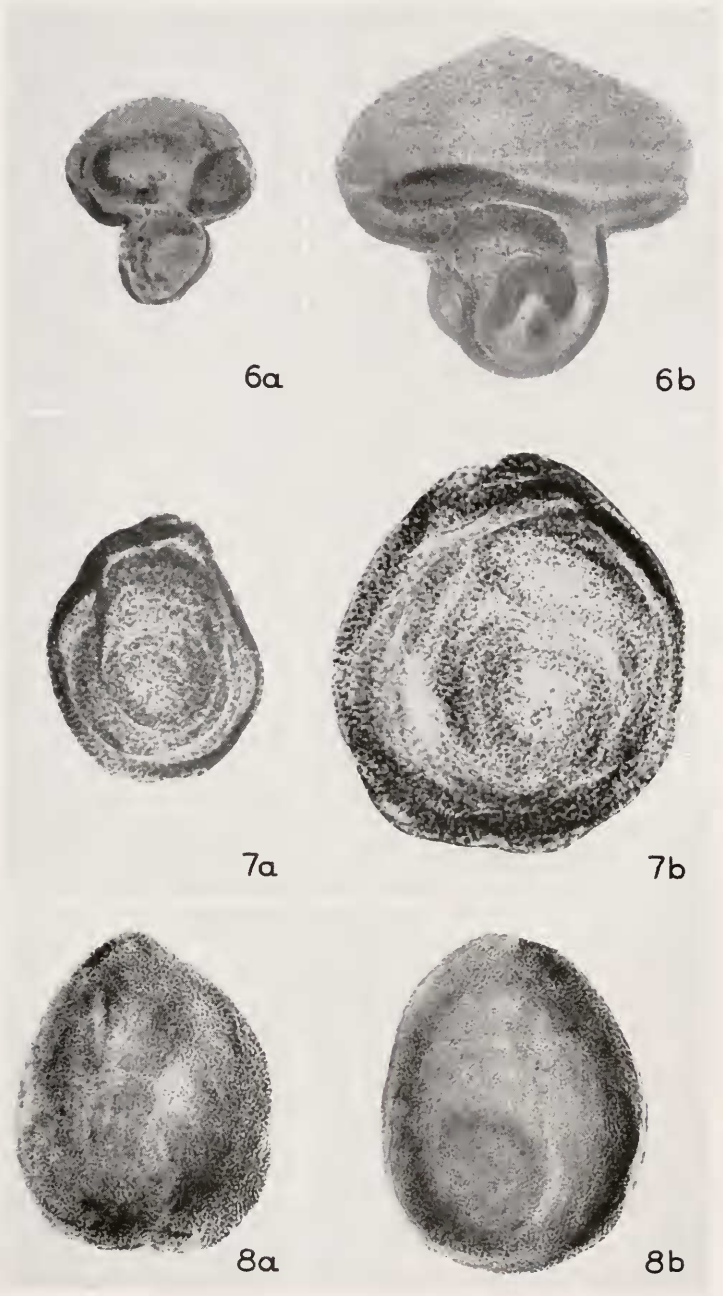

PLATE II

slightly larger and in the last three pairs they were somewhat larger in the virilis hosts. The fact that the discs may be larger in melanogaster female as well as in virilis female hosts indicates that there is no significant difference between the environment of both host species.

e. Growth test in male and female hosts in the presence of ring glands.

It has been shown before that organ discs grow larger in female hosts than in male hosts. The question now arises, how the growth of equal discs is affected when they are under the influence of the same number of ring glands in both environments. For this, two partner discs of a virilis leg pair were transplanted, one into a virilis male and the other into a virilis female host, while at the same time each of the two hosts in two of such pairs received five ring glands, and in two other pairs four ring glands from mature larvae. In dissecting the two pairs with five ring glands three days, and the two pairs with four ring glands four days after the operation, it was found that the discs in the female hosts of all pairs were larger than their partner discs in the male hosts. However, in contrast to the earlier experiments, where the discs were grafted alone, into male and female hosts, both discs in this experimental combination had grown. Moreover, the discs in the female hosts had become much larger in the presence of ring glands than discs which had grown in female hosts without ring glands.

f. Growth tests in different host species in the presence of ring glands.

If the experiments where virilis partner leg discs are transplanted into two hosts of different species (melanogaster male and virilis male) are repeated, but each host receives in addition two ring glands from mature larvae, then the results obtained are quite different. The transplanted organ discs are much larger in the melanogaster hosts (Figures 7*a* and 7*b*). This was observed in nine out of 12 pairs four of which were dissected three days, six four days and two six days after the operation. Only in three pairs left for four days in the host were the discs found to be alike in both hosts. Moreover in another pair, where one partner disc was transplanted into a virilis male host together with five ring glands, and the other partner disc into a melanogaster male host together with four ring glands, it was found again that the disc in the melanogaster host was much larger. Finally, in two additional pairs in which one partner disc was transplanted together with four ring glands, into a virilis male host, and the other partner into a melanogaster male host together with only two ring glands, it was again observed that, three days after the operation, the discs in the melanogaster hosts were much larger than their partners in the virilis hosts. Thus two ring glands in melanogaster hosts are able to induce more growth in the test organs than four ring glands in virilis hosts. Since in all these cases virilis ring glands were used as grafts we witness the peculiar fact that virilis ring glands are more effective in a foreign than in their own species environment.

II. The activating system

Experiments described in this section are designed to further the understanding of the ring gland action.

a. The effect of different numbers of ring glands on organ growth.

We have seen that organ discs transplanted into adult male hosts are able to grow only when under the influence of simultaneously transplanted ring glands. It remained to be shown, however, as to how many ring glands are actually needed to assure maximum growth of the organ disc. For this, eye discs of virilis larvae of equal size were transplanted from virilis male hosts while in addition, each of these hosts received a different number of ring glands from mature larvae. The results of the experiments are summarized in Table II,

TABLE II

Effect of different number of ring glands on eye growth.

Experiment	Number of ring glands	Transplant remains in hosts for three days		Transplant remains in hosts for four days	
		Number of cases	Size of eyes	Number of cases	Size of eyes
A	0	4	no growth	4	no growth
B	1	4	larger than A	4	larger than A
C	2	4	larger than B	4	larger than B
D	4	4	larger than C	4	larger than C
E	8	4	larger than C	4	larger than C

where it can be seen that two ring glands produce a greater growth effect in the test organs than one ring gland, but that the effect of four or eight ring glands is the same as that produced by only two ring glands. This evidence is further supported by experiments in which the two partners of single virilis leg pairs subjected to the influence of a different number of ring glands in virilis male hosts are compared. Table III summarizes the results obtained from six such

TABLE III

The effect of different numbers of ring glands on leg growth.

Number of ring glands compared	0 and 1	2 and 4	4 and 8	2 and 8	2 and 8	2 and 8
Days leg pairs remain in hosts	4	4	4	3	9	9
Size of leg pairs	larger in ring gland host	same in both hosts	same in both hosts	same in both hosts	same in both hosts	same in both hosts

pairs, showing again that the growth of the test organ is the same whether two, four or eight ring glands are present (Figures 8*a* and 8*b*). In summarizing the results, we must conclude that one ring gland is apparently unable to raise the hormone concentration in the adult male fly to a level high enough to assure maximal growth in the test organs. Yet the hormone concentration produced by two ring glands must have reached the level of saturation as far as the growth

of the test organ is concerned, since more than two ring glands have no greater effect than only two ring glands.

Evidence that the number of ring glands is also of importance for the time of imaginal differentiation of the test organ is provided by the following experiments. Melanogaster eye discs of equal size were transplanted into adult melanogaster female hosts, (*a*) alone, (*b*) together with two ring glands, (*c*) together with three ring glands, (*d*) together with four ring glands. All hosts were dissected eight days after the operation. It was found that in the hosts without ring glands the eye discs had grown, but were still white and showed no sign of imaginal differentiation (seven cases). In the hosts with two ring glands, reddish pigmented regions could be seen in the transplanted discs, showing that the ring gland had brought about pigment differentiation in the eye disc (six cases). The eyes in hosts with three ring glands (four cases) and in hosts with four ring glands (four cases), had developed to the same stage of pigmentation. In a further set of experiments melanogaster eye discs were transplanted alone into melanogaster female hosts but left for 16 days (two cases), 17 days (one case), and 22 days (one case), in the hosts before they were dissected. By this time pigmentation had also started in these eyes but was in a much less advanced stage of development. This is indicated by the slight yellow coloration, as contrasted with the reddish color, developed in eyes grown in hosts with ring glands. These experiments show that color development can take place in eye discs transplanted into female hosts without the support of ring glands, but that it is much less rapid than in female hosts in the presence of ring glands. The onset and degree of eye pigmentation in the presence of two, three or four ring glands in female hosts is about the same. Thus as in the experiments where the effect of different numbers of ring glands on the early growth of the eye discs was tested we find that for the later processes of differentiation also two ring glands produce the maximal effect.

b. The effect of ring glands of different age on organ growth.

Until now we have studied only the effects of mature ring glands, that is, of ring glands from larvae shortly before pupation. It remains to be seen, however, whether there is any difference in the effects produced by younger or older ring glands. Single pairs of virilis leg discs were thus transplanted, one partner alone and the other partner together with ring glands, into two virilis male hosts. The virilis ring glands used for each pair were of different age. In this way progressively younger ring glands were tested as to their effect on the growth of the organ discs. In two series of this kind, each host received three ring glands, in a third series, four ring glands. All pairs in the three separate series were dissected five days after the operation and the growth of the disc in each pair compared. The results of the experiments are summarized in Table IV, where it can be seen that ring glands of all ages, even when coming from larvae as young as five and a half days before pupation, i.e. young first instar larvae, are able to promote growth in the transplanted leg test disc. This proves that the ring gland can produce its growth hormone during the entire larval period of the animal. Whether there may be any interruption in the hormone production of the ring gland during this period, as the few negative cases might indicate (see Table IV), is not known, and needs further investigation.

c. Differences in hormone production of young and old ring glands.

The question as to the amount of hormone produced by young and older ring glands was tested in the following way: melanogaster leg discs of an average diameter of 13 units were divided into three lots. One set of legs was transplanted

TABLE IV

The effect of ring glands of various age on organ growth. In all cases the test organ remained for five days in the host. Positive indicates that the leg partner in the host with ring glands is larger than its partner in the host without ring glands; while negative indicates that both leg partners in the two hosts compared are of the same size.

Number of ring glands transplanted	Ring gland donor. Age in days before pupation	Ring gland donor, larval stage	Result
3	before pupation	3	positive
3	before pupation	3	positive
3	1	3	positive
3	1	3	positive
3	2	3	positive
3	2	3	positive
3	3	⅔	negative
3	3	⅔	negative
3	4	2	negative
3	4	2	positive
3	5	1	positive
3	before pupation	3	positive
3	before pupation	3	positive
3	1	3	negative
3	1	3	positive
3	3	3	positive
3	4	2	positive
3	4	2	negative
3	5	1	positive
3	5	1	positive
4	1	3	positive
4	1	3	positive
4	2	3	negative
4	2	3	positive
4	3	3	positive
4	4	2	positive
4	4	2	negative
4	5	1	positive
4	5	1	positive
4	5½	1	positive
4	5½	1	positive

into melanogaster females alone, the legs of the second set into melanogaster females each together with one melanogaster ring gland from an old larva shortly before pupation, and the legs of the third set into melanogaster females each together with one two-day younger melanogaster ring gland. Three days after the operation the hosts of these three groups were dissected and the transplanted

leg discs measured and compared. The legs in the hosts without ring glands (eight cases) had grown to an average diameter of 16 units. The legs in the second group with one young ring gland (five cases) were found to average 21 units in diameter and in the last group with one old ring gland, the average diameter of the legs (four cases) was 24 units. It was noticed, moreover, that the legs in the last group had begun to evaginate, which was not the case in the other two groups.

Now one may test, although in a somewhat different way, the amount of hormone produced. If it is true, as the above mentioned experiments indicate, that one young ring gland produces less hormone than one old ring gland, one might expect equal discs transplanted at the same time, and left long enough in the adult host, to be advanced further in their metamorphosis in the presence of old ring glands than in the presence of the same number of younger ring glands. In such an experiment we use the state of metamorphosis rather than differences in growth as an indicator for the hormone concentration. In order to elucidate this point, melanogaster eye discs of equal age were transplanted into adult hosts, some with four ring glands from larvae shortly before pupation and others with four one day younger ring glands. Eight days after the operation the hosts were dissected and the discs compared. The eyes in hosts with four ring glands had developed yellow-red pigment (four cases) while the eyes in hosts with four younger ring glands were much less advanced in their differentiation. Although they had grown extensively in the presence of the younger ring glands, they were still white, showing no trace of pigmentation (four cases). From these two groups of experiments we may thus conclude with reasonable certainty that young ring glands produce less hormone than old ring glands.

d. Species differences in ring glands.

Qualitatively the ring glands of virilis and melanogaster are the same. This has been shown many times in experiments where the action of virilis or melanogaster ring glands has been tested as to its effect on the growth and differentiation of melanogaster or virilis organ discs. The question whether there is any quantitative difference in the amount of hormone output during a given time between the ring glands of these two species is, however, not so clear. Since the ring gland of virilis is larger than that of melanogaster one might expect it to produce more hormone. If quantitative differences between melanogaster and virilis ring glands are present, they are at least not large, as the following experiment indicates. Equal melanogaster eye discs were transplanted into adult melanogaster female hosts together with two ring glands from a melanogaster larva shortly before pupation (three cases) and together with two virilis ring glands from larvae shortly before pupation (three cases). The dissection of these cases eight days after the operation showed that in the hosts with melanogaster ring glands, one eye disc had developed slight yellow pigment and two eye discs yellow-red pigment. In the hosts with two virilis ring glands, two eye discs had become slightly yellow and one eye disc yellow-red pigmented. We thus observe about the same amount of development under the influence of the same number of melanogaster or virilis ring glands, indicating that there is no difference in the quantity of hormone production between the ring glands of these two species tested.

e. The time of action of ring glands in adult hosts.

The question as to how long transplanted ring glands in adult hosts continue to produce hormone was tested as follows: virilis ring glands from larvae shortly before pupation were transplanted into adult virilis males. The glands were left in these hosts for a certain length of time, then dissected out and re-transplanted into a second adult host together with one partner disc of a leg pair. The other leg partner was transplanted alone into another virilis male host. After several days the pair of hosts was dissected, the growth of the leg discs compared and the ring gland grafts recovered. The recovered ring glands were now grafted for the third time into a virilis male host together with new test leg discs, the partners of which were again transplanted as the growth control into virilis male hosts alone. Several days later the pairs were dissected, the leg discs compared and the retransplantation procedure of the recovered ring glands using new test organs and new hosts repeated once or twice more. Since it was not easy to recover such a small organ as the ring gland from the body of the adult fly, two or four ring glands were usually transplanted together into one host. If one ring gland was lost in the dissection, the remaining ring glands could be used to continue the test. Table V shows the results of these experi-

TABLE V

The time of action of ring glands in adult hosts. (For explanation see text.) Positive indicates that the retransplanted ring gland has stimulated the growth of the test organ.

Experiment	A	B	C	D	E
Days ring gland remains in first host	6	6	10	10	22
Days ring gland retransplanted together with test organ remains in second host. Condition of test organ	5 positive	6 positive	6 positive	6 positive	6 positive
Days ring gland retransplanted together with test organ remains in third host. Condition of test organ	4 positive	5 positive		6 positive	
Days ring gland retransplanted together with test organ remains in fourth host. Condition of test organ		5 positive		4 negative	
Days ring gland retransplanted remains in fifth host. Condition of test organ		3 positive			

ments. Each of the five columns (A to E) represents one case of successive re-transplantation of the same original glands. Now, as it can be clearly seen from Table V, it was found that ring glands after being in adult hosts for 22 days, during which time they had been three times retransplanted and found to be active, were still active in a fourth transplantation (Table V B). Similarly, ring glands which were left for 22 days in one host before they were tested in a second host for their activity were still able to induce growth in the test

organ (Table V E). From these experiments we must conclude that ring glands transplanted into adult hosts secrete their hormone continuously for a long time.

III. The reacting system

It has to be realized that the various kinds of organ discs may differ as to their responsiveness towards the same hormone level. We may also expect differences in the responsiveness between old and young organ discs. Experiments which investigate these possibilities are presented in the following.

a. The differentiation capacity of different discs in adult hosts.

In comparing the first growth effect of such organs as eye and leg discs, one finds both very responsive to the hormone of the ring gland. Even in female hosts without ring glands, which must be considered the least favorable environment, the growth effect of both discs is considerable. Thus there seems to be no appreciable difference in the responsiveness between leg and eye discs. Yet when one compares the further development of these discs in the adult environment a marked difference between these organs becomes evident. One finds the leg discs able to differentiate in the adult host to imaginal completion under the influence of ring glands, but not the eye discs, which never continue their differentiation beyond the first stages of pigmentation. The leg discs in their final state of differentiation show typical imaginal characteristics; i.e. dark brown chitinized leg segments covered with chitinized hairs and bristles and with a blackish chitinized end claw on the distal tarsus segment. In the eye discs on the other hand, we find that the pigment is the only component which differentiates to an appreciable extent. There is however some doubt whether even pigmentation reaches its final imaginal stage. The development of hairs, bristles, lenses, or the darkening of the chitinous eye parts has never been observed in eye grafts. Although a more detailed histological examination of these partially developed eyes is still missing, there can be no doubt that differentiation is incomplete, since it would have been easy to detect chitinous structures in total mounts if they were present. It was found, moreover, that the anlage of the genital apparatus, when transplanted into adult hosts, is unable to differentiate at all, even in melanogaster female hosts in the presence of four ring glands, thus in an environment where the ring glands are most effective. Independent of the time these genital discs remain in the host, they never develop beyond a stage corresponding to the stage the discs would have reached in normal development at the time of puparium formation. In Table VI we have summarized a number of experiments in which different organ discs were transplanted together with ring glands into different adult hosts. Only such cases are recorded where the grafts were left for more than seven days in the host. We find, for example, that a melanogaster leg disc in a melanogaster female host in the presence of only one ring gland has already differentiated imaginal characters eight days after the operation, while a melanogaster eye disc in the same host in the presence of as many as four ring glands has developed only to the stage of pigment formation 19 days after the operation. Since about the same amount of pigment is present in melanogaster eyes which were left for eight days in female hosts together with two ring glands, it follows that the differentiation in the 19-day old eye has not progressed much beyond that observed in the eight-day old eye.

Thus in a melanogaster female host environment under the influence of two or more ring glands, the eye discs reach their limit of differentiation about ten days after the operation. As far as the genital discs are concerned, we find them to grow somewhat beyond their stage of transplantation. Their growth, however,

TABLE VI

The differentiation capacity of different discs in adult hosts.

Number of cases	Transplanted organ	Donor	Host	Number of transplanted ring glands	Days transplant remains in host	Result
1	♀ genital disc	viril.	viril. ♀	0	8	no clear change
1	♀ genital disc	mel.	mel. ♀	0	9	no clear change
2	♀ genital disc	viril.	viril. ♀	0	17	prepupal
2	♀ genital disc	mel.	mel. ♀	0	20	prepupal
2	eye	mel.	mel. ♀	0	16	light yellow spots; no hairs
1	eye	mel.	mel. ♀	0	17	light yellow spots; no hairs
1	eye	mel.	mel. ♀	0	22	light yellow spots; no hairs
3	eye	mel.	mel. ♀	1	9	red yellow spots; no hairs
1	eye	mel.	mel. ♀	1	16	reddish spots; no hairs
1	leg	mel.	mel. ♀	1	9	brownish hairs and chitin diff.
1	♀ genital disc	viril.	mel. ♀	4	7	little growth; prepupal
1	♀ genital disc	viril.	viril. ♀	4	8	little growth; prepupal
1	♀ genital disc	viril.	mel. ♀	4	9	little growth; prepupal
1	♀ genital disc	viril.	mel. ♂	4	9	little growth; prepupal
2	♀ genital disc	mel.	mel. ♀	4	9	little growth; prepupal
2	♀ genital disc	viril.	viril. ♂	2	10	little growth; prepupal
2	♀ genital disc	viril.	viril. ♂	4	10	little growth; prepupal
1	♀ genital disc	viril.	viril. ♀	4	10	little growth; prepupal
1	♀ genital disc	viril.	viril. ♂	4	13	little growth; prepupal
1	♀ genital disc	viril.	mel. ♂	4	13	little growth; prepupal
1	♀ genital disc	mel.	mel. ♀	4	14	little growth; prepupal
1	♀ genital disc	viril.	viril. ♀	4	17	little growth; prepupal
6	eye	mel.	mel. ♀	2	8	yellow red spots; no hairs
4	eye	mel.	mel. ♀	3	8	yellow red spots; no hairs
4	eye	mel.	mel. ♀	4	8	yellow red spots; no hairs
1	eye	mel.	mel. ♀	4	16	large reddish spots; no hairs
1	eye	mel.	mel. ♀	4	19	large reddish spots; no hairs
1	eye	mel.	mel. ♀	3	23	large reddish spots; no hairs
1	leg	viril.	viril. ♂	3	9	large; no hairs
4	leg	mel.	mel. ♀	3	9	brownish hairs and chitin diff.
1	leg	viril.	mel. ♀	4	9	brownish hairs and chitin diff.
2	leg	viril.	viril. ♀	3	10	hairs diff.; still white
1	leg	viril.	viril. ♀	3	10	brownish hairs and chitin diff.
1	leg	viril.	viril. ♂	3	12	hairs diff.; still white
2	leg	viril.	viril. ♀	3	13	brownish hairs and chitin diff.
1	leg	viril.	viril. ♂	3	14	brownish hairs and chitin diff.
3	leg	viril.	viril. ♂	3	16	brownish hairs and chitin diff.

is very much slower than that of leg or eye grafts, even in their early growth effect. They never surpass, as said before, a prepupal stage, although they may be as long as 17 days in a female environment under the influence of four ring glands.

Table VII

Comparison of the differentiation capacity of different discs from the same donor larvae transplanted together with ring glands into one adult host.

Experiment	Transplant	Donor	Host	Number of transplanted ring glands	Transplant remains in host	Condition of organ at dissection		
						Leg disc	Eye disc	Genital disc
A	leg eye disc	mel.	mel.	3	8	large, first white hairs	red yellow spots; no hairs	—
B	leg eye disc	mel.	mel.	3	8	large, first white hairs	red yellow spots; no hairs	—
C	leg eye disc	mel.	mel.	3	8	large, first white hairs	red yellow spots; no hairs	—
D	leg eye disc	viril.	mel. ♀	4	14	brownish hairs and chitin	large white; no hairs	—
E	leg eye genital disc	viril.	viril. ♀	3	8	large, no hairs	large white; no hairs	little growth; prepupal
F	leg eye genital disc	viril.	viril. ♀	3	11	large, no hairs	large white; no hairs	little growth; prepupal
G	leg eye genital disc	viril.	viril. ♀	3	11	large with white hairs	large white; no hairs	little growth; prepupal
H	leg eye genital disc	viril.	viril. ♀	3	11	large with white hairs	large white; no hairs	little growth; prepupal
I	leg eye genital disc	viril.	viril. ♀	3	11	brownish hairs and chitin	large white; no hairs	little growth; prepupal
J	leg eye genital disc	viril.	mel. ♀	4	9	large with white hairs	large white; no hairs	little growth; prepupal
K	leg eye genital disc	viril.	mel. ♀	4	13	large with white hairs	large white; no hairs	little growth; prepupal
L	leg eye genital disc	viril.	mel. ♀	4	13	large with white hairs	large white; no hairs	little growth; prepupal
M	leg eye genital disc	viril.	mel. ♀	4	13	large with white hairs	large, yellow spots, no hairs	little growth; prepupal
N	leg eye genital disc	viril.	mel. ♀	4	11	large with white hairs	large white; no hairs	little growth; prepupal
O	leg eye genital disc	viril.	mel. ♀	4	14	brownish hairs and chitin	large white; no hairs	little growth; prepupal

The capacity of the three imaginal discs tested to differentiate in adult hosts in the presence of ring gland thus decreases in the order leg, eye and genital disc. This can be demonstrated conclusively by a somewhat different experiment, as follows: a leg, eye and genital disc from the same donor larva were transplanted into a single host, together with ring glands. In this way all the three discs are under the influence of the same number of ring glands in the same host environment and can thus be compared more directly than in the previous experiments. Fifteen such cases are shown in Table VII, where it can be seen that the differentiation capacity of eye, leg and genital discs of the same animal differs markedly under the same hormonal conditions.

Before closing this section, one further point of importance should be mentioned. It has been found that there are also differences in the differentiation capacity in the various regions of the same organ disc. The clearest example of this phenomenon is provided in the differentiation of the leg disc. While it is true that leg discs are able to differentiate to imaginal completion in adult hosts, this statement must be modified somewhat, because it applies only to the distal leg disc portions. It is known that the larval leg disc not only includes the presumptive tissue of the actual adult leg, but also some material which gives rise to the ventral body wall in the nearest neighbourhood of the leg. This proximal portion of the leg disc never differentiates completely, while the distal leg portions consisting of femur, tibia and tarsus develop to imaginal completion. The differentiation capacity of the leg parts seems to increase in a proximal distal direction, since we find the tarsus segments always to be the first structures which become imaginal, and only if the discs are left longer in the host do we find tibia and femur completely differentiated. However, we have not been able to compel the proximal leg disc portions to become completely differentiated, although the discs have remained for a considerably longer time in the host than that needed for the complete differentiation of the distal leg parts. A very similar situation prevails in the eye discs, where we find that material which gives rise to pigment cells is able to express its differentiation tendencies, resulting in the formation of well-differentiated red pigment, while the material destined to form hairs or lenses is unable to differentiate to any great extent. Moreover, there seem to be regional differences in the eye for pigment formation also, for we observe most frequently that only certain eye regions are pigmented while others are still white. It seems possible that this last phenomenon might be correlated with the position of the graft in the host as well as with the arrangement of folding of the developing eye, which in turn may affect the oxygen supply in the different eye regions, and thus promote or inhibit, as the case may be, the oxidation of pigment.

b. Differences between young and old discs.

Young and old discs in the same hormonal environment differ as to their time of differentiation. This has been shown by the following experiment. Young and old leg or eye discs were transplanted simultaneously into single adult hosts together with one or more ring glands. The grafts were left in the host for not less than nine days; they were then dissected and compared. In all cases it was found that the older graft was further differentiated than the younger graft (see Table VIII).

TABLE VIII

Differences between young and old discs, transplanted together into one host.

Experiment	Transplant	Donor	Host	Number transplanted ring glands	Days transplant remains in host	Condition of organ at dissection	
						Old disc	Young disc
A	young and old eye	mel.	mel. ♀	1	9	large reddish spots; no hairs	large white; no hairs
B	young and old eye	mel.	mel. ♀	1	9	large reddish spots; no hairs	large white; no hairs
C	young and old leg	mel.	mel. ♀	1	9	large, with white hairs	large; no hairs
D	young and old leg	mel.	mel. ♀	1	9	large, with white hairs	large; no hairs
E	young and old leg	viril.	viril. ♀	3	10	large, with white hairs	large; no hairs
F	young and old leg	viril.	viril. ♀	3	10	large, with white hairs	large; no hairs
G	young and old leg	viril.	viril. ♀	3	10	brownish hairs and chitin	large; no hairs
H	young and old leg	viril.	viril. ♀	3	13	brownish hairs and chitin	large; no hairs
I	young and old leg	viril.	viril. ♀	3	13	brownish hairs and chitin	yellowish hairs and chitin

c. The responsiveness of organs of different species.

If one compares (Tables VI and VII) the final developmental condition of melanogaster eyes with virilis eyes which were left for the same length of time in an approximately equal adult environment, one observes that the melanogaster eyes have developed further than the virilis eye discs. This indicates a difference in the competence of virilis and melanogaster eyes to respond to the same hormonal conditions. The same indication is seen in another set of experiments where virilis leg discs were transplanted into virilis female hosts together with three ring glands (five cases) and melanogaster leg discs (four cases) into virilis females together with three ring glands. Eleven days after the operation two melanogaster legs had developed hairs which were, however, still white; the two other melanogaster legs were completely differentiated, showing yellow-brown chitinous structures and hairs. Three of the virilis legs were without any hairs, one had hairs but was white, and one was completely differentiated. Although these observations speak for the assumption that virilis organs respond with greater difficulty to the same hormonal environment, there is one further point to be taken into consideration. Virilis and melanogaster differ in their time of development. The larval as well as the pupal period of virilis is much longer than that of melanogaster. Therefore it is possible that in the above-mentioned experiments, leg discs of unequal age were compared, especially since no accurate record was made of the exact age of the donor discs. The observed difference in the time of differentiation between virilis and melanogaster organs may thus not really reflect species differences but rather age differences. Even if we assume that the organs in question were of the same age, this would mean only that they were alike in their chronological age but not in their physiological age. In the light of these considerations it becomes evident that it is rather difficult in experiments of this kind to be quite sure whether any discrepancies in the time of differentiation between discs of two species are caused by species-specific responses or age effects.

DISCUSSION

The present investigations have brought forward a number of pertinent facts concerning the relationship between hormone actions and tissue competence in

the development of Drosophila organ discs. It has been shown that the organ discs depend for their growth as well as for their imaginal differentiation upon the action of the ring gland which functions as a gland of internal secretion. As judged from their effect on test organs, young ring glands produce qualitatively the same hormone as ring glands of a mature larva. The quantity of hormone produced by young ring glands is presumably less than that produced by old ring glands. Equal ring glands differ as to their effect in hosts of different species and in the two sexes of the same species. The amount of organ growth during a given time and the speed at which differentiation proceeds depends upon the number of ring glands, i.e. on the amount of hormone available as well as on the competence of the organs to respond. Different organ discs as well as discs of different ages and different regions within the same organ disc differ as to their competence to respond. These facts reflect very clearly the highly relative nature of conditions which find their expression in the processes of growth and differentiation. They show that we cannot ascribe absolute values to either organ competence or hormone concentration but rather that we have to measure one in terms of the other.

We have now to consider in more detail certain aspects of the problem of hormone-controlled growth and differentiation which have arisen in the course of these investigations. For this it seems best to discuss separately the principal points in question, and after we have estimated their value to try to fit them into the framework of the general concept.

A. Relationship between hormone concentration and effective level.

Of particular interest is the observation that organ discs transplanted into adult female hosts are able to grow even in the absence of ring glands. Since we know that the growth of the transplanted organ is under the control of the ring gland hormone we might assume that female hosts, in contrast to male hosts, either produce or have stored some hormone. We know further that two ring glands have the same effect as four glands. In the presence of two or four ring glands, therefore, the environment of either female or male hosts must be considered saturated with hormone as far as the growth of the organ is concerned. We should thus expect the hormonal environment of female and male hosts to be the same, i.e. saturated when both hosts are supplied with five ring glands each. Consequently, we should also expect the growth response of identical organs grown in such a saturated male and female environment to be the same. This, however, is not the case, as the experiments show (see p. 42). The discs in the female environment grow much better than their partners in the male environment, although both were in a hormone-saturated environment. This suggests that the ring gland hormone does not act directly but, rather, indirectly by the intervention of some factors in the host. Limited by the lack of further knowledge on this point we might assume for the time being that the ring gland hormone establishes what might be called an "effective level" in the host, which in turn is responsible for the various reactions of the test organs. This assumption is supported by the fact that we observe similar differences in the reaction of the test organs under the influence of the same number of ring glands in different species. In these cases, too, a different growth effect is produced when the hormone concentration has saturated the environment.

For example, we find that two ring glands in melanogaster hosts have a greater effect on the growth of the test organ than four ring glands have in virilis hosts. However, there is a definite relationship between the hormone concentration and the effectiveness of this level. We find that a low hormone concentration produced by one ring gland is unable to raise the host level to its most effective state, while the hormone concentration produced by two ring glands already brings the level to its highest state of effectiveness. Although the effective level cannot be raised above a certain threshold even when higher hormone concentrations, i.e. more ring glands, are used, its peak effectiveness is nevertheless higher in females than in males and in melanogaster than in virilis. Yet there is no apparent difference in the effective level of the females in these two species when tested without ring glands. The difference between the species becomes evident only when their levels are elevated by the ring gland hormone. Whether the low effective level of female hosts is caused by the presence of a small amount of hormone is as yet still obscure, but of course possible. In any event, it seems unlikely that some hormone is stored, since in this case we would expect that the stored hormone would gradually decrease as the flies become older. The experiments show, however, that young and old flies are equally affected. Now, when one follows the thread of implications connecting these various points it becomes evident that one may obtain different effective host levels either by varying up to a certain point the hormone concentration, or by varying the host animals. For example, the lowest effective level prevails in female hosts without ring glands. The effective level is somewhat higher in male hosts with one ring gland. In melanogaster and virilis male hosts with two or more ring glands, the effective level is lower than in the virilis female hosts with two or more glands, while in a melanogaster female with two or more ring glands the effective level is highest. If in the following we speak of hormone concentration, it should be understood that we always refer to a host level of a certain effectiveness, produced by a definite concentration of ring gland hormone in a definite host.

It is characteristic that organ discs are unable to grow in adult male hosts without the support of ring glands. The male host environment was thus considered neutral. Now we know only that the adult male environment is neutral as far as the larval discs are concerned. Whether pupal organs which are presumably much more responsive than larval organs are also unable to develop in male hosts is not known so far. Actually it would be very difficult to prove that such an environment is neutral in an absolute sense, i.e. for all larval as well as all pupal tissues. If we should find, for example, that pupal discs, but not larval discs, would develop in adult male hosts and from this conclude that the pupal discs have attained the capacity of independent development, this conclusion could well be erroneous. We must take into consideration that the effective level in the male hosts, although too low for the growth of the larval discs, might well be high enough to assure the development of the highly responsive pupal discs. This argumentation brings us directly to one further aspect of the problem. In an earlier paper (Bodenstein, 1939a) it was shown that eye discs of young pupae continue their development when transplanted into larvae the anterior parts of which were cut off by means of a ligature. From these experiments the conclusion was drawn that pupal eye discs, which already had been stimulated by the differentiation-promoting hormone, are able to

develop independently in an environment lacking the differentiation stimulus. At the time these experiments were performed we did not know that the ring gland is the source of the hormone which promotes differentiation, nor that this hormone is produced in younger larval stages. The larval host therefore was expected to contain no differentiation hormone. Although the source of the hormone supply in these earlier experiments was cut off by the ligature, and thus no hormone coming from the ring gland could have reached the transplant, it is highly probable that enough hormone was left in the rear part to account for the continued development of the transplanted organ. Since we must assume that even a very low hormone concentration is sufficient to affect the very responsive older eye discs, this experiment does not prove the independent development of the pupal eye. Ephrussi (1943) has recently performed a similar experiment. He transplanted eye discs from mature larvae into the abdomen of young larvae and observed that these discs developed synchronously with the host organs. However, in another series of experiments where he transplanted eye discs of one-day old pupae into young larvae he found the transplanted eyes to develop heterochronously. In these cases the transplanted pupal eye had already formed red pigment while the hosts were still in their larval stage. These experiments also do not prove the independence of eye development, for the hormone concentration in the young larvae, although not high enough for the differentiation of the larval host organs, might have been sufficient for the differentiation of the pupal eye. In the light of these considerations, it is very difficult indeed to be sure whether one is dealing with dependent or independent development. Again we are confronted with the fact that development is not the reflection of absolute conditions, but is highly relative indeed; it is the expression of a very delicate balance between the activating and reacting systems involved.

B. The effective level and tissue competence.

It takes about eight days for a leg disc to differentiate to imaginal completion in a very effective female environment obtained by a hormone concentration produced by two or more ring glands, while in normal development in the presence of only one ring gland the leg disc completes its differentiation in four days. This shows that the effective level in the normal pupal environment must be much higher than that of the most effective adult environment. This low level in the adult environment is very fortunate for the understanding of the responsive capacity of the test organs, since it has brought to light real differences in the responsiveness of different test organs and of different regions within identical organs. For example, if we compare different discs as to their capacity to differentiate, we find in the most effective adult environment only the distal parts of leg discs are able to complete their imaginal differentiation, while under the same conditions, eye discs differentiate only partially and genital discs not at all. These differences in the responsive capacity of the different discs are not detectable if we grow them in a pupal environment under the influence of a very effective level. For, if we transplant legs, eyes and genital discs into larvae shortly before pupation, all these discs become mature in complete synchrony with the host organs and there seems to be no difference between them as far as their responsiveness is concerned. We have demonstrated that young and old

discs grown in a highly effective adult environment differ in their time of onset of differentiation. The young leg discs begin and complete their differentiation considerably later than older leg discs. When finally even the young discs have attained imaginal character they are of approximately the same size as the older discs. In other words, the young discs grow to a certain size before their differentiation leading to imaginal completion begins. This seems in disagreement with the results of earlier experiments (Bodenstein, 1939b) where it was found that young eye discs transplanted into older larval hosts differentiated prematurely, that is, before they had reached their full larval size, and as a consequence were finally smaller than normal eyes. When we recall that the effective pupal level is much higher than even the most effective level in an adult environment, we realize how we can explain the observed discrepancies between the results of our earlier and present experiments. Obviously, the pupal level is high enough to induce premature differentiation in the young organ while the adult level is able only to promote growth in the young organ. Only after the young disc in the adult environment has grown to a certain stage and has thereby become more readily responsive is the low effective adult level able to induce differentiation also into the young disc. Experiments in which the responsiveness of young and old salivary glands was tested (Bodenstein, 1943a in press) yield the same results. These experiments show that differentiation takes place only when both organ-responsiveness and effective level together attain a sufficient value. The difference between the responsive capacity of young and old organ discs is also clearly demonstrated by experiments (Bodenstein, 1939a and b) in which very young eye discs were transplanted into larvae shortly before pupation. In these cases the very young eye discs were only partly differentiated at the time the host emerged, although they had been under the influence of the very effective pupal level. This shows that even the very effective pupal environment is unable to bring about complete differentiation in test organs which are very young and hence possess a very low responsive value.

If we list the different organ discs as to their capacity to differentiate in the most effective adult environment, we find them arranged in the following order: legs, larval salivary glands, eyes, adult salivary glands and genital discs. Under the influence of the same effective adult level we thus find that the value for the differentiation response is highest in the leg disc and lowest in the genital and adult salivary gland discs, while the values for the other discs tested fall between these extremes. However it seems that the larval skin is more readily responsive than all the organ discs, as the following experiments by Hadorn and Neel (1938) indicate. The authors transplanted ring glands into young larvae of the early third instar and found that under the influence of the ring gland grafts puparium formation took place prematurely, yet these prepupae failed to develop further. This indicates that the larval skin is very responsive indeed, since it responded to the increased hormone level with puparium formation, before the organ discs were able to respond and hence failed in their differentiation.

Viewing the specific results of the investigations we conceive the following general picture of the mode of action of the ring gland in the development of Drosophila. The larval ring gland of Drosophila is an organ of internal secretion which produces its hormone during the entire larval period. This hormone controls the growth of organ discs during larval life. In the course of larval

development the ring gland becomes larger and produces more hormone, while at the same time the responsiveness of the organ discs increases as they grow older. When the hormone concentration and the responsiveness of the organ discs have reached a certain value, the ring gland hormone controls imaginal differentiation also. The evagination of the organ discs is the first indication that they have reached a differentiation phase. In normal development this stage is reached at the time of pupation. Pupation is thus nothing more than the first step in the process of differentiation. The kind of organ response, i.e. whether the organ discs respond with growth or differentiation to the ring gland hormone depends upon a definite relationship between hormone concentration and organ responsiveness. It is very probable that the ring gland hormone has no direct effect on the reacting organ systems, but that it rather acts indirectly through the intervention of some as yet unknown mechanism. If these conclusions deduced from experimental results are correct, it should follow that extirpation of the ring gland in the larval stage should prevent the growth of the organ anlagen. This experiment, technically not possible in Drosophila, has actually been performed by Burtt (1938) on Calliphora larvae, with the result that the growth of the organ disc was arrested in larvae which had their ring glands removed. These experiments then provide further evidence that the ring gland hormone controls not only differentiation but also the processes of organ growth during the larval period. The general interpretation of the problem under discussion is in contrast to Hadorn's view; he maintained that only ring glands from mature larvae produce hormone and that this hormone controls solely the processes of puparium formation, but has no effect on the growth or differentiation of the organ discs. On the basis of our experimental evidence, Hadorn's conception seems to be no longer tenable.

Summary

A variety of organ discs of Drosophila was transplanted together with or without ring glands into the body cavity of adult flies and their developmental behavior in their new surroundings studied. The specific results of these investigations are briefly summarized as follows:

1. Organ discs transplanted into adult male hosts cease to develop but remain alive presumably indefinitely. The transplanted discs do not lose their developmental potencies, although development may be arrested for a long time.

2. Organ discs transplanted into adult male hosts will grow and finally differentiate to imaginal completion when under the influence of simultaneously transplanted ring glands.

3. Organ discs transplanted into adult female hosts continue their development at a very slow rate even in the absence of ring glands.

4. There is no difference in the organic environment of different species as far as the development of test organs is concerned. If however, different host species are provided with the same number of ring glands it is found that the ring glands have a greater effect on the development of the test organs in melanogaster than in virilis hosts.

5. Ring glands of all larval ages, even from larvae only 12 hours old, are able to induce growth in the transplanted test organ.

6. The amount of hormone produced by young larval ring glands is less than that produced during the same time interval by ring glands of mature larvae.

7. Different organ discs differ as to their capacity to differentiate in adult hosts under the influence of ring glands.

8. Different regions within the same organ disc also differ as to their differentiation capacity.

9. Under the same hormonal environment it takes the young organ discs a considerably longer time to complete differentiation than it takes the old organ discs.

10. The ring gland hormone, apparently, does not affect the reacting organ directly, but acts rather through the intervention of some as yet unknown factors in the host.

11. The kind of organ response, that is, whether the organ disc responds with growth or differentiation to the ring gland hormone depends upon the relationship between hormone concentration and organ responsiveness.

12. The problem of growth and differentiation in the development of Drosophila is discussed. It is pointed out that development is not the reflection of absolute conditions but that it is highly relative indeed; it is the expression of a very delicate balance between the activating and reacting systems involved.

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